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Preoperative chemoradiotherapy in locally advanced rectal cancer: correlation of a gene expression-based response signature with recurrence

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Abstract Preoperative chemoradiotherapy is recommended for locally advanced rectal cancer (UICC stage II/III). We recently demonstrated that responsive and nonresponsive tumors showed differential expression levels of 54 genes. In this follow-up study, we investigated the relationship between this gene set and disease-free (DFS) and overall survival (OS). Pretherapeutic biopsies from 30 participants in the CAO/ARO/AIO-94 trial of the German Rectal Cancer Study Group were analyzed using gene expression microarrays. Statistical analysis was performed to identify differentially expressed genes between recurrent and nonrecurrent tumors and to correlate these changes with disease recurrence and outcome. After a median follow-up of 59 months, seven of eight patients with recurrent disease was a nonresponder, and one responsive tumor recurred. Response to chemoradiotherapy was significantly correlated with an improved DFS (log rank $P = 0.028$), whereas OS did not differ significantly ($P = 0.11$). Applying a class comparison analysis, we identified 20 genes that were differentially expressed between recurrent and nonrecurrent tumors ($P < 0.001$). Analyzing the first two principal components of the 54 genes previously identified to predict response, we observed that this response signature correlated with an increased risk of cancer recurrence. These data suggest that the genetic basis of local response also affects the genetic basis of tumor recurrence. Genes that are indicative of nonresponse to preoperative chemoradiotherapy might also be linked to an increased risk of tumor recurrence.

1. Introduction

Gene expression profiling has been extensively applied to study colorectal tumors, and comparisons of primary tumors with associated mucosa samples or precursor lesions have been published [1–7]. Subsequently, stage-specific signatures were described [8–12], and profiles of recurrence and prognosis [13–18] or response to chemotherapy [19,20] were derived for colon cancers. Because prognostic or predictive signatures are still lacking for locally advanced rectal cancers (International Union Against Cancer UICC stage II/III) [21], we explored whether transcriptional profiling might unveil signatures indicative of therapeutic response to preoperative chemoradiotherapy and survival.

As a result of the recently published CAO/ARO/AIO-94 trial of the German Rectal Cancer Study Group [22], preoperative 5-fluorouracil-based chemoradiotherapy is now recommended for UICC stage II/III rectal cancer in Germany, large parts of the rest of Europe, and the United States [23]. Tumor response is heterogeneous, however, and ranges from complete response to resistance [24]. We recently demonstrated for a subset of patients treated within this clinical trial that pretherapeutic gene expression profiling might be useful for prediction of response to preoperative chemoradiotherapy [25]. From a clinical perspective, however, there is considerable debate on how to reliably assess and define response, and it remains to be determined how tumor response relates to the individual patient's prognosis.

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Nonetheless, it is of considerable relevance to establish predictive markers for response, and ultimately, survival.

After a median follow-up of 59 months, we correlated chemoradiotherapy-induced T-level downsizing with disease-free survival (DFS) and overall survival (OS). Additionally, we evaluated the relationship of expression changes of the identified set of 54 genes and disease recurrence.

2. Materials and methods

2.1. Patient samples and clinical treatment

All 30 patients (age: 60.7 ± 8.2 years) participated in the CAO/ARO/AIO-94 trial of the German Rectal Cancer Study Group [22], and were treated at the Department of General and Visceral Surgery, University Medicine, Göttingen, Germany. Preoperative chemoradiotherapy, surgical treatment (including total mesorectal excision [26]) and histopathologic workup were standardized as part of the clinical trial. Only patients with uT3 ($n = 29$) and uT4 ($n = 1$) adenocarcinomas located within 16 cm from the anocutaneous verge were included in this study. The experimental design is summarized in Figure 1, and the clinical data are summarized in Table 1.

2.2. Histopathological staging

Histopathological staging was performed according to the tumor–node–metastasis classification of the UICC [27], and resected specimens were assessed according to established protocols [28,29]. Cases with resection margins (oral, aboral, lateral, and circumferential) free of vital tumor cells within a minimum distance of 1 mm were classified as R0 tumor resection.

2.3. Response classification

Response to preoperative chemoradiotherapy was previously defined as downsizing of the primary rectal cancer by comparing the uT-category (determined by endorectal ultrasound) with the histopathologically assessed ypT-status [25]. Previously, we had demonstrated that the endoscopic assessment of the T-category correlated very well with the histopathological diagnosis [30]. A reduction of tumor infiltration by at least one T-category resulted in classification as responsive (T-level downsizing). Furthermore, a reduction of the pretherapeutic UICC-category compared to the histopathologic UICC-category by at least one category was defined as UICC downstaging. Histopathologic tumor regression grading was determined based on

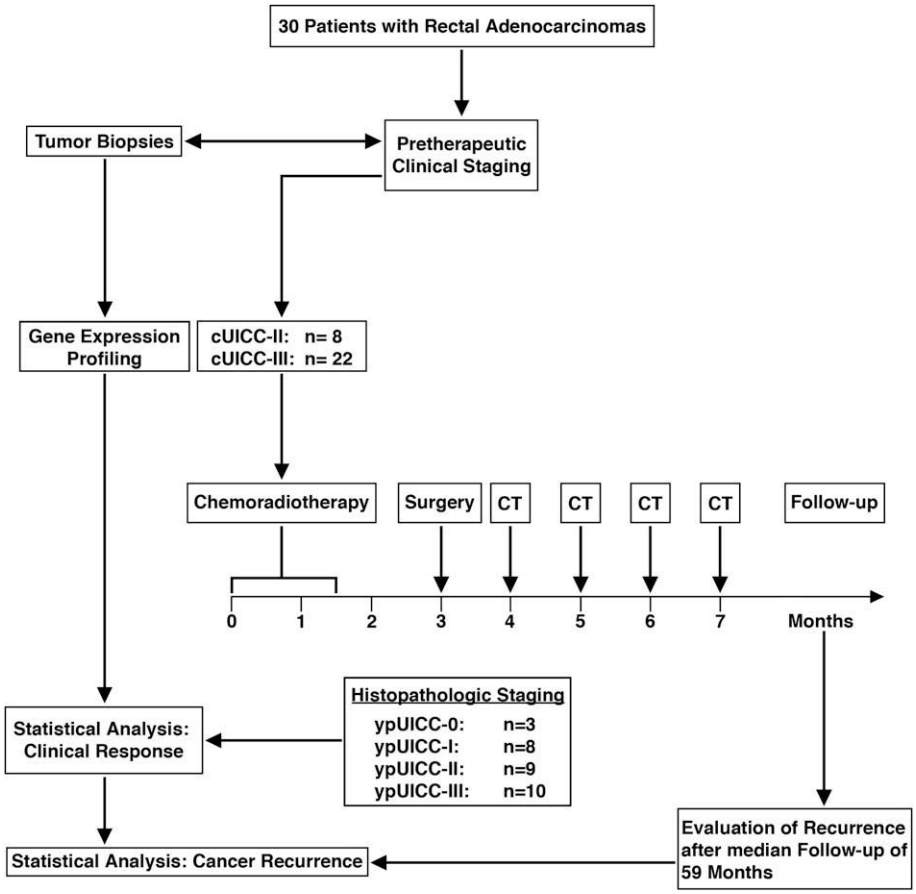

Fig. 1. Presentation of study design, clinical treatment and follow-up. cUICC refers to pretherapeutic clinical staging of locally advanced rectal cancers, ypUICC to histopathological assessment of the resected specimens after preoperative chemoradiotherapy; CT, postoperative chemotherapy.

Table 1
Clinical data for 30 patients in the CAO/ARO/AIO-94 trial of the German Rectal Cancer Study Group

Tumor sample	uT	ypT	T-level downsizing	ypN	ypN total	ypN infiltrated	cUICC stage	ypUICC stage	UICC downstaging	Regression grading	Recurrence	DFS	OS	Status
P1	3	0	+	0	18	0	II	0	+	4	—	64	64	alive
P2	3	0	+	0	27	0	III	0	+	4	—	58	58	alive
P3	3	0	+	0	16	0	II	0	+	4	—	54	54	alive
P4	3	2	+	0	22	0	III	I	+	3	—	74	74	alive
P5	3	1	+	0	20	0	II	I	+	3	—	65	65	alive
P6	3	2	+	0	24	0	III	I	+	3	—	60	60	alive
P7	3	1	+	0	18	0	II	I	+	3	—	58	58	alive
P8	4	3c	+	1	19	3	III	III	−	3	—	74	74	alive
P9	3	2	+	0	16	0	II	I	+	2	—	68	68	alive
P10	3	3b	−	1	30	1	III	III	−	3	—	66	66	alive
P11	3	3b	−	0	15	0	III	II	+	3	liver + peritoneum	3	7	dead
P12	3	3b	−	0	8	1	II	III	−	3	—	78	78	alive
P13	3	3b	−	1	27	1	III	III	−	3	—	53	53	alive
P14	3	4a	−	1	19	1	III	III	−	2	lung	35	66	alive
P15	3	3b	−	0	28	0	III	II	+	2	—	65	65	alive
P16	3	3a	−	0	21	0	III	II	+	2	—	59	59	alive
P17	3	3b	−	1	19	2	III	III	−	2	lung	51	74	alive
P18	3	4a	−	1	21	2	III	III	−	3	local + peritoneum	35	57	dead
P19	3	3c	−	0	24	0	III	II	+	1	—	74	74	alive
P20	3	3c	−	0	16	0	II	II	−	1	liver + cerebrum	5.5	21	dead
P21	3	3c	−	0	17	0	III	II	+	1	—	59	59	alive
P22	3	3a	−	0	14	0	III	II	+	1	lung	12	68	alive
P23	3	4a	−	1	22	1	III	III	−	1	—	74	74	alive
P24	3	2	+	0	16	0	II	I	+	3	—	52	52	alive
P25	3	2	+	0	17	0	III	I	+	2	—	50	50	alive
P26	3	3	−	0	20	0	III	II	+	2	liver	17	48	alive
P27	3	2	+	2	14	5	III	III	−	3	—	48	48	alive
P28	3	Tis	+	1	17	1	III	III	−	3	lymph node metastasis	49	53	alive
P29	3	3c	−	0	15	0	III	II	+	2	—	53	53	alive
P30	3	2	+	0	12	0	II	I	+	2	—	41	41	alive

Abbreviations: cUICC, clinical UICC stage; DFS, disease-free survival in months reflecting the interval from R0 resection to cancer recurrence; OS, overall survival in months reflecting the interval between surgery and any death including cancer-specific survival; Tis, tumor in situ; UICC, International Union Against Cancer; uT, pretherapeutic T-category determined by endorectal ultrasound; ypN infiltrated, number of infiltrated lymph nodes; ypN total, total number of analyzed lymph nodes; ypN, lymph node status by histopathological assessment; ypT, T-level determined by histopathological assessment after preoperative chemoradiotherapy; ypUICC, post-treatment UICC stage.

a semiquantitative five-point classification system as proposed earlier [31].

2.4. Clinical follow-up

All patients were followed at 3-month intervals for the first two years, and then at 6-month intervals, according to the CAO/ARO/AIO-94 trial design [22]. Disease-free survival was defined as the interval between potentially curative (R0) tumor resection and local or distant cancer recurrence. Data for patients who were alive and remained without local, distant, or both local and distant cancer recurrence at the last observation, as well as patients who died without relapse, were censored for the survival analysis. Overall survival was defined as the interval between R0 resection and death due to any cause, including cancer-specific death.

2.5. Gene expression profiling

Gene expression profiling was performed as previously described [25]. An initial set of 23 tumors was hybridized to cDNA microarrays (9,984 features), and an additional set of seven tumors was hybridized to oligonucleotide microarrays (22,231 features).

2.6. Statistical analysis

2.6.1. DFS and OS

The Kaplan–Meier survival estimates method was applied to calculate OS and DFS. The differences in DFS and OS between patients with and without T-level downsizing were determined by the log-rank test; results with a P-value of <0.05 were considered significant. The analyses were performed using R statistical software, version 2.3.0 (http://www.r-project.org).

2.6.2. Class comparison

To determine genes differentially expressed between patients with disease recurrence and those without, we performed a class comparison analysis using the *BRB-ArrayTools* package developed at the Biometric Research

Branch of the National Cancer Institute (Bethesda, MD) [32]. The two-sample *t*-test with a randomized variance model [33], and a stringent statistical significance threshold of $P < 0.001$, was applied. A permutation test was performed to obtain the significance of finding a given number of genes satisfying the *P*-value criteria if there was no relationship between recurrence and gene expression [32].

2.6.3. Gene expression signatures and risk of recurrence

To test the hypothesis that the previously identified set of genes is correlated with DFS and OS, we used the first and second principal components of the expression of these 54 genes to create a two-dimensional plot of the variations in the molecular signatures of the different samples. The principal components are weighted sums of the expression levels of these differentially expressed genes, which are chosen to maximize the variance. For consistency, we analyzed only those 23 tumors that were hybridized to cDNA arrays.

3. Results

3.1. Patient characteristics

Twenty-two patients with rectal adenocarcinomas were diagnosed with cUICC stage III, and eight patients with cUICC stage II (Table 1). According to the clinical trial design, all operations were performed by four experienced and well-trained surgeons. Locally curative (R0) tumor resection was achieved for all patients. Surgical procedures included 13 low anterior resections (43.3%) for tumors with a median pretherapeutic tumor location within 9 cm above the anocutaneous verge (mean, 8.8 cm ± 3.2 cm). Seventeen abdominoperineal resections (56.7%) were performed due to a median tumor location within 3 cm above the anocutaneous verge (mean, 3.4 cm ± 2.3 cm). In median, 18.5 lymph nodes (mean, 19.1 ± 4.9 lymph nodes) were investigated to determine the nodal status (ypN0, $n = 20$; ypN1, $n = 9$; ypN2, $n = 1$) and the UICC stage (ypUICC stage 0, $n = 3$; ypUICC I, $n = 7$; ypUICC II, $n = 10$; ypUICC III, $n = 10$).

Comparison of the pre- and post-therapeutic staging revealed that T-level downsizing was achieved in 14 of 30 patients (46.7%); eight cases were downsized by one T-level, two cases by two T-levels, and four cases by three T-levels (Table 1). Only patients who showed at least one T-level reduction were considered responders (P1–P9, P24, P25, P27, P28 and P30). UICC downstaging was achieved in 19 of 30 patients (63%): in 13 cases by one UICC-stage, in 5 cases by two UICC-stages, and in 1 case by three UICC-stages.

All tumors showed a certain degree of tumor regression, and we observed three tumors with a TRG 4 (complete regression), 13 tumors with a TRG 3, 9 tumors with a TRG 2, and 5 tumors with a TRG 1. We believe that the most reasonable stratification would be to divide the tumors into complete responders (TRG 4) and nonresponders or partial responders (TRG 0–3). Because this was not possible, due to unequal sample distribution, no further analysis was attempted.

3.2. Recurrence rate, DFS, and OS

After a median follow-up of 59 months (mean, 58 months with 95% confidence interval (28.6, 87.4) using standard error of the mean), eight patients developed recurrent disease (P11, P14, P17, P18, P20, P22, P26, and P28). One patient (P18) developed local recurrence (3.3%) associated with simultaneous peritoneal metastases (35 months after R0 resection of the primary tumor). Although no patient died during preoperative chemoradiotherapy or within the first 30 days postoperatively, three patients (10%) died due to distant metastatic cancer progression at 7 months (P11), 21 months (P18), and 57 months (P20) after tumor resection (Table 1). We therefore calculated a DFS of 73% (22/30), and an OS of 90% (27/30; data not shown). Figures 2a and 2b show the Kaplan–Meier curves for DFS and OS, respectively, with T-level downsizing used as the surrogate endpoint for response. Response to chemoradiotherapy was significantly correlated with DFS ($P = 0.028$), but not with OS ($P = 0.11$). However, UICC-downstaging was not associated with DFS ($P = 0.11$) or OS ($P = 0.29$) (data not shown). Furthermore, quality assessment for total mesorectal excision did not correlate with survival data (data not shown).

3.3. Comparison of clinical response and recurrence

When we observed that seven of the eight patients who developed recurrent disease belonged to the nonresponsive group, we interpreted this as strong indication that recurrence is intimately connected to the absence of T-level downsizing. To quantify this relationship, the right-tailed Fisher's exact test was used to compute the probability of all patients with recurrence belonging to the nonresponse group if recurrence and response were independently distributed (i.e., not connected to each other). Because we observed a *P*-value of 0.030 for the null hypothesis that response and recurrence are not connected, it can be rejected. We therefore concluded that there is a positive correlation between response to chemoradiotherapy, defined as downsizing of the T-category, and recurrence.

3.4. Class comparison analysis

The class comparison analysis between recurrent and nonrecurrent cancer samples revealed 20 genes that were differentially expressed at a *P*-value of <0.001 (Table 2). Notably, 7 of these 20 genes were also present in the list of 54 genes that we have identified to be differentially expressed between responsive and nonresponsive tumors [25]. Nonetheless the probability of finding 20 genes by chance at this level of significance is high ($P = 0.079$), which might be further complicated by the fact that the

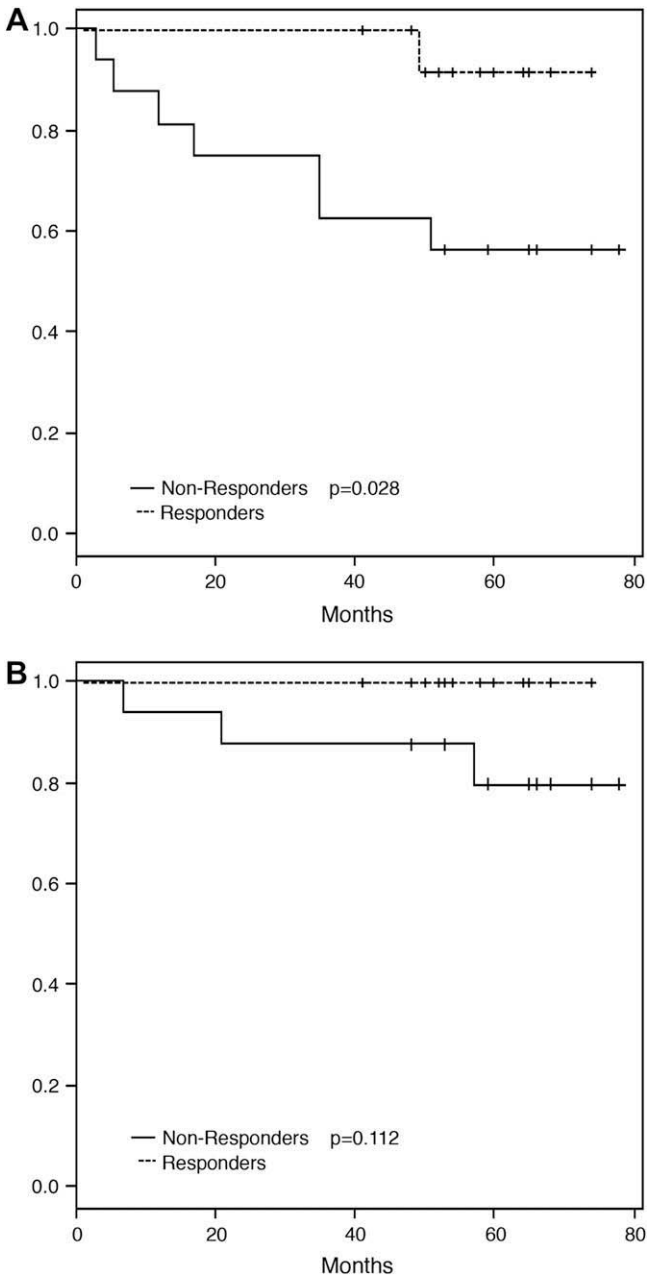

Fig. 2. Disease-free (A) and overall (B) survival data for all 30 patients treated with preoperative chemoradiotherapy. (A) Responders, as measured by T-level downsizing, showed a significantly better disease-free survival than did nonresponders. (B) No statistically significant difference in overall survival was observed between responders and nonresponders.

numbers of patients in the two groups (recurrence and nonrecurrence) are unequal (Table 1).

3.5. Correlation of gene expression signatures and risk of recurrence

We previously used cDNA microarrays to demonstrate that a set of 54 genes was differentially expressed between responsive and nonresponsive rectal cancers (set 1, $n = 23$). This was further validated for an independent set of tumor samples using oligonucleotide microarrays (set 2, $n = 7$).

In the present investigation, for consistency, we analyzed only those 23 tumors that were hybridized to the cDNA microarrays. In this data set, all six patients with recurrence belonged to the group of nonresponders. When we displayed the molecular signature of these 54 genes in a principal component plot (Fig. 3), it became obvious that all recurrent tumors (P11, P14, P17, P18, P20, and P22) were positioned farther away from the responsive, nonrecurrent tumors (P1–P9) than from the nonresponsive, nonrecurrent tumors (P10, P12, P13, P15, P16, P19, P21, and P23). This demonstrated that recurrence occurred only in patients whose tumors were (correctly) classified to be the most distant from the boundary between responders and nonresponder to preoperative chemoradiotherapy when response was measured as T-level downsizing.

4. Discussion

The CAO/ARO/AIO-94 trial of the German Rectal Cancer Study Group demonstrated that preoperative 5-fluorouracil-based chemoradiotherapy is superior to postoperative chemoradiotherapy in UICC stage II/III rectal cancer in terms of local control and of acute and long-term toxicity [22]. This study showed that the 5-year cumulative incidence of local cancer recurrence was 6% for patients randomly assigned to preoperative chemoradiotherapy, compared with 13% in the group of patients treated with postoperative chemoradiotherapy ($P = 0.006$). The 5-year OS rates, however, did not differ significantly. Furthermore, two recent phase III trials demonstrated that preoperative 5-fluorouracil-based chemoradiotherapy is more effective than radiotherapy alone with respect to local control, but not in terms of 3-year DFS and OS [34,35]. Based on these studies, preoperative chemoradiotherapy is now considered standard of care in most countries in Europe and in large parts of the United States [23].

Recently, we demonstrated for a subset of the patients treated within the CAO/ARO/AIO-94 trial that gene expression profiling might be useful for pretherapeutic prediction of local response to preoperative chemoradiotherapy [25]. Fifty-four genes showed significantly different expression levels ($P < 0.001$) between responders and nonresponders (measured by T-level downsizing). There is considerable debate, however, over the most meaningful method for the assessment of tumor response. Although response actually correlated with clinical outcome in the data set presented here, a recent investigation concluded that response to preoperative 5-fluorouracil-based chemoradiotherapy does not linearly translate into improved survival [24].

The present investigation of possible correlation of the expression pattern of these 54 genes with clinical outcome (DFS and OS) allowed an assessment of the relationship between gene expression signatures and survival data from

Table 2
Twenty genes differentially expressed ($P < 0.001$) between recurrent and nonrecurrent rectal adenocarcinomas

Parametric *P*value	Geometric mean of ratios		Fold difference of geometric means	Gene symbol	Description	Clone	UG cluster	Map
	Recurrence	Nonrecurrence						
0.0000496	1.144	0.523	2.188	*ZNF609* (alias *KIAA0295*)	KIAA0295 protein	IncytePD:3520727	Hs.155979	15q22.1
0.0000744	1.686	0.791	2.133		ESTs	IncytePD:1398814	Hs.355960	12
0.0000869	2.070	0.951	2.178	*ZFP106**	zinc finger protein 106	IncytePD:2757735	Hs.15220	15q14
0.0001145	1.215	0.451	2.693	*ELL2*	ELL-related RNA polymerase II, elongation factor	IncytePD:1281473	Hs.98124	5q14.3
0.0001212	1.272	0.640	1.989	*RAB11FIP5* (alias *KIAA0857*)	KIAA0857 protein	IncytePD:3770939	Hs.24557	2p13~p12
0.0001301	3.497	1.202	2.909		ESTs	IncytePD:4003773	Hs.131511	2
0.0001412	2.208	1.035	2.132	*KTN1**	kinectin 1 (kinesin receptor)	IncytePD:3736760	Hs.211577	14q22.1
0.0001665	3.817	1.112	3.431	*ITGA8*	integrin, α8	IncytePD:3085610	Hs.91296	10p13
0.0001678	3.584	1.597	2.244	*AKAP13*	A kinase (PRKA) anchor protein 13	IncytePD:1563055	Hs.301946	15q24~q25
0.0001925	1.942	0.804	2.416	*DDX17*	DEAD/H box polypeptide 17	IncytePD:1750553	Hs.349121	22q13.1
0.0001981	1.529	0.664	2.303	*RBM25** (alias *S164*, *RED120*)	S164 protein	IncytePD:2047730	Hs.180789	14q24.3
0.0003126	1.477	0.714	2.068	*CHD2*	chromodomain helicase DNA binding protein 2	IncytePD:523797	Hs.36787	15q26
0.0004637	1.456	0.585	2.488	*AP3D1**	adaptor-related protein complex 3, δ1 subunit	IncytePD:1301192	Hs.75056	19p13.3
0.0004843	2.198	1.159	1.897	*PAK1**	p21/Cdc42/Rac 1-activated kinase 1	IncytePD:2632434	Hs.64056	11q13~q14
0.0006413	2.004	0.866	2.315		*Homo sapiens*, clone IMAGE:3458340, mRNA	IncytePD:2208874	Hs.405949	17
0.0006743	3.333	1.739	1.917		*Homo sapiens* cDNA FLJ10158 fis, clone HEMBA1003463	IncytePD:3144018	Hs.104627	3
0.0007382	1.047	0.540	1.939	*MLL**	myeloid/lymphoid or mixed-lineage leukemia	IncytePD:1692195	Hs.199160	11q23
0.000778	1.330	0.678	1.963	*PPP1R10**	protein phosphatase 1, regulatory subunit 10	IncytePD:2314555	Hs.106019	6p21.3
0.0008452	1.364	0.726	1.880		ESTs	IncytePD:2382190		
0.0009194	1.764	1.075	1.640	*ELF2*	E74-like factor 2	IncytePD:2834326	Hs.82143	4q28

* Seven genes overlapped with the 54-gene set previously identified as differentially expressed between responsive and nonresponsive tumors [25].

patients treated within a phase III clinical trial. We observed that T-level downsizing was significantly correlated with DFS (Fig. 2), because seven of eight patients with metastatic disease belonged to the group of nonresponders. We therefore concluded that T-level downsizing actually represents a surrogate clinical endpoint that might allow response prediction for a subset of rectal cancer patients receiving preoperative 5-fluorouracil-based chemoradiotherapy, followed by postoperative chemotherapy. However, T-level downsizing did not correlate with OS, which might be due to the low rate of cancer-related deaths (10%) within the follow-up period of 59 months.

The class comparison analysis revealed 20 genes that were differentially expressed between patients with recurrence and those without at a *P*-value of <0.001 (Table 2), although the probability of finding 20 genes by chance at this level of significance is high. A plausible explanation for this discrepancy may be the uneven distribution of recurrent and nonrecurrent patients (6 vs. 17 tumors, respectively).

Seven genes of this recurrence signature overlap with our previously established response signature. The most interesting of these genes in the context of therapy resistance is probably *PAK1*, the p21 protein (Cdc42/Rac)-activated kinase 1 gene. *PAK1* represents a target for the small

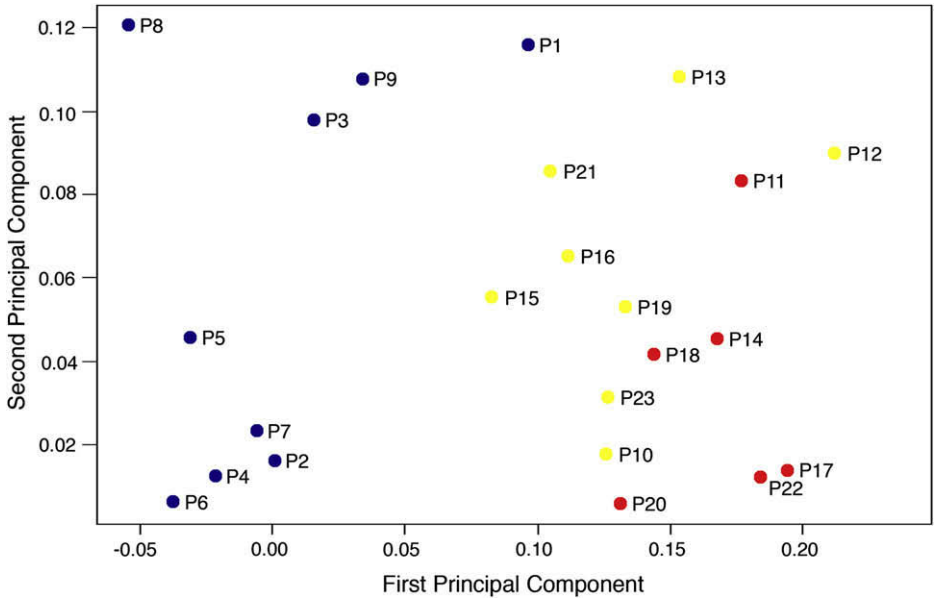

Fig. 3. Principal component plot of the 54 genes that were previously identified using cDNA microarrays to be differentially expressed between 9 responsive and 14 nonresponsive tumors. None of the responders (blue: P1–P9) developed disease recurrence. Six of the nonresponders had tumor recurrence (red: P11, P14, P17, P18, P20, and P22); the remaining eight nonresponders did not develop metastatic disease (yellow: P10, P12, P13, P15, P16, P19, P21, and P23).

GTP-binding proteins Cdc42 and Rac, and functions as regulator of cell motility, cell morphology and cell proliferation, and nuclear signaling. Recent data indicate that amplification of *PAK1*, which activates the estrogen receptor, is a predictor of recurrence and tamoxifen resistance in breast cancers [36,37]. Furthermore, abrogation of *PAK1* function restored sensitivity of renal cell cancer cells to chemotherapy [38].

The mixed lineage leukemia gene, *MLL*, encodes a DNA-binding protein. It is involved in recurrent chromosomal translocations in acute leukemias, and, interestingly, often predicts a poor prognosis [39]. A recent study identified specific miRNAs that were upregulated as a consequence of this translocation [40].

For most of the other genes, the possible connection to resistance of cancer cells to chemoradiotherapy and, subsequently, tumor recurrence, remains to be determined. *ZFP106* encodes for the zinc finger protein 106, which has been shown to play a role in testis development [41]. Kinectin 1 represents an endoplasmic reticulum membrane protein, encoded by the *KTN1* gene. Interacting with other microtubule-associated proteins such as the ATPase kinesin, it helps to move vesicles along the microtubules. Recent studies indicated that kinectin might be involved in the regulation of protein synthesis [42,43]. *RBM25* (alias *S164*, *RED120*) encodes for an SRm-interacting protein. It is supposed to bridge ribonucleoprotein complexes and represents a splicing coactivator [44]. The gene for the δ1 subunit of the adaptor-related protein complex 3, *AP3D1*, has been shown to be a key component required for transporting enveloped viral particles from the Golgi apparatus to the cell surface [45]. *PPP1R10* encodes a protein that regulates the protein phosphatase 1, which is involved in mitosis exit and chromosome decondensation [46].

In summary, these data suggest that the genetic basis of local response to preoperative chemoradiotherapy is not independent of the genetic basis of tumor recurrence. Our results therefore indicate not only that pretherapeutic profiling may separate responders and nonresponders, but also that the set of 54 genes might be representative of local response as well as an increased risk for recurrence.

These preliminary results require validation in an independent and larger patient population [47]. With integration into a Clinical Research Unit entitled ‘‘Biological Basis of Individual Tumor Response in Patients with Rectal Cancer’’ (KFO 179), we have initiated prospective profiling of tumor samples from patients enrolled in the ongoing CAO/ARO/AIO-04 trial of the German Rectal Cancer Study Group, which compares standard preoperative 5-fluorouracil-based chemoradiotherapy against an intensified protocol (5-fluorouracil + oxaliplatin + radiation).

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